

Role of Dermal Melanocytes in Cutaneous Pigmentation of Stasis Dermatitis

: A Histopathological Study of 20 Cases

Stasis dermatitis is an itchy, scaly, and hyperpigmented condition of the lower leg due to venous insufficiency. Hemosiderin and/or melanin have been considered responsible for the brown pigmentation. However, there are not sufficient histopathologic studies. In this retrospective study the hospital records and biopsy slides of 20 patients were reviewed to determine the pathogenetic mechanisms of brown pigmentation in stasis dermatitis. Fifteen were men (75%) and 5 were women (25%) with a mean age of 46.2 ± 8.2 yr (18-76), mean age at onset of 43.4 ± 18.0 yr (17-73), and a mean duration of the disease 2.8 ± 2.5 yr (0.25-10). All patients had varicose vein and complained of pruritus. On histopathologic evaluation, two cases out of 20 (3 skin biopsy specimens from 25 samples) showed dermal melanocytes containing melanin, and incontinence of melanin pigment was observed in 5 cases, which indicates that melanin pigments from epidermis could contribute to cutaneous pigmentation in stasis dermatitis. However, the existence of dermal melanocytes in two cases cannot be explained because normally the dermis contains no melanocytes. Further studies concerning the role of iron or inflammatory cytokines on the development of dermal melanocytes should be conducted.

Key Words : Venous Insufficiency; Pigmentation, Iron, Hemosiderin; Melanins; Melanocytes

Daehwan Kim, Won Hyoung Kang

Department of Dermatology, Ajou University School of Medicine, Suwon, Korea

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Address for correspondence

Won Hyoung Kang, M.D.
Department of Dermatology, Ajou University School of Medicine, 5 Wonchon-dong, Paldal-gu, Suwon 442-721, Korea
Tel : +82.31-219-5190, Fax : +82.31-219-5189
E-mail : www@madang.ajou.ac.kr

INTRODUCTION

Stasis dermatitis occurs secondary to venous hypertension of the lower extremities. This disorder has a predilection for middle-aged to elderly females with venous incompetence or an inadequate calf pump, and any patients who have deep vein thromboses. Patients with long-standing venous insufficiency and lower extremity edema may develop pruritic, erythematous, scaly papules and plaques on their lower legs, often in association with brown pigmentation and hair loss. Dilated or varicose vein is a frequent predisposing factor of stasis dermatitis. Histopathologic findings include fibrosis, new blood vessel formations, and hemosiderin deposition in dermis (1-3).

Cutaneous pigmentation in stasis dermatitis often causes cosmetic problems for the patients. Hemosiderin and/or melanin have been considered responsible for the brown pigmentation (4, 5). However, there are not sufficient histopathologic studies to explain the pathogenesis of the discoloration in stasis dermatitis. Melanin is the major determinant of the normal skin color. Other contributing chromophores are oxyhemoglobin (red), deoxygenated hemoglobin (blue), and ca-

rotene (yellow-orange). It is the presence or absence of melanin in melanosomes that is responsible for the skin color. Melanin pigment is synthesized in the specialized cytoplasmic organelles called melanosomes, which contain the principal pigment-synthesizing enzyme, tyrosinase. Melanocytes, specialized dendritic cells derived from the neural crest, are the sole site of melanin formation (6). However, there has been no report clarifying the role of melanin or melanocytes in the production of cutaneous pigmentation in stasis dermatitis.

In the present study, we tried to identify the pigments or cells responsible for the discoloration in stasis dermatitis by using various special staining techniques for melanin, hemosiderin, macrophages, and melanocytes with skin biopsy samples from 20 patients with stasis dermatitis.

MATERIALS AND METHODS

We selected twenty patients with typical clinical and histopathological findings of stasis dermatitis, who visited the dermatological department of Ajou University Medical Center between 1994 and 2001. Clinical analysis and review of

medical records were performed regarding age, sex, age of onset, duration of the disease, characteristics of individual lesions, and other associated dermatologic or systemic diseases.

Light microscopic examinations were undertaken using 25 skin biopsy specimens from 20 patients. Four-micrometer-thick sections (paraffin blocks) were stained with hematoxylin and eosin (H&E). However, H&E stain has its limitations in differentiating melanin from hemosiderin and melanocytes from siderophages. In order to differentiate melanin from hemosiderin, we performed Fontana-Masson stains for melanin pigment, Perls' potassium ferrocyanide stains for Fe³⁺ (Prussian blue reaction) (7), and potassium ferricyanide stains for Fe²⁺ (Turnbull blue reaction) (8). For the differentiation between melanocytes and macrophages, additional paraffin sections were obtained and immunohistochemical staining using antibodies (clone: NKI-beteb) to glycoprotein 100 (GP 100) and antibodies (clone: KP1) to CD68 were performed (Table 1). The monoclonal anti-melanocyte antibody NKI-beteb recognizes a 100 kDa (pre) melanosome-associated antigen (glycoprotein) and reacts with melanomas, nevocellular nevi, and normal melanocytes (9, 10). CD68 is the lysosomal marker of monocytes/macrophages (11). These procedures were performed using the standard avidin-biotin peroxidase com-

plex technique. However, each staining method mentioned above shows only one component (melanin, hemosiderin, melanocytes, or siderophages) in a section when performed alone. Therefore, triple stain (immunohistochemical staining to CD68 combined with Fontana-Masson stain, and Perls' stain) was performed.

RESULTS

Out of 20 patients, 15 were men (75%) and 5 were women (25%) with a mean age of 46.2 ± 8.2 yr (18-76). The mean age at onset of stasis dermatitis was 43.4 ± 18.0 yr, ranging from 17 to 73. The mean duration of stasis dermatitis was 2.8 ± 2.5 yr (0.25-10) before diagnosis.

Clinical findings

All of the selected patients showed pruritic, erythematous, scaly papules and plaques with dark-brown to black pigmentation on the lower legs (Fig. 1A). All patients had varicose vein. Two patients (patient #4, #13) had deep vein thrombosis, and one (#1) had long-standing (8 yr) thrombophlebitis. One (#8) patient had psoriasis. Hypertension (#3, #6, #13), angina (#8), old myocardial infarction with mitral valve stenosis (#13), stroke (#10), liver cirrhosis with esophageal varix (#3), Behcet's disease (#4, #12), iatrogenic Cushing disease (#5), rheumatoid arthritis (#9), acute myelogenous leukemia (#18), and diabetes (#3, #8) were found in 10 patients.

Table 1. Immunohistochemical stains

Antibodies	Clone	Dilution	Source
GP 100	NKI-beteb	1:20	Monosan
CD 68	KP1	1:200	Novocastra

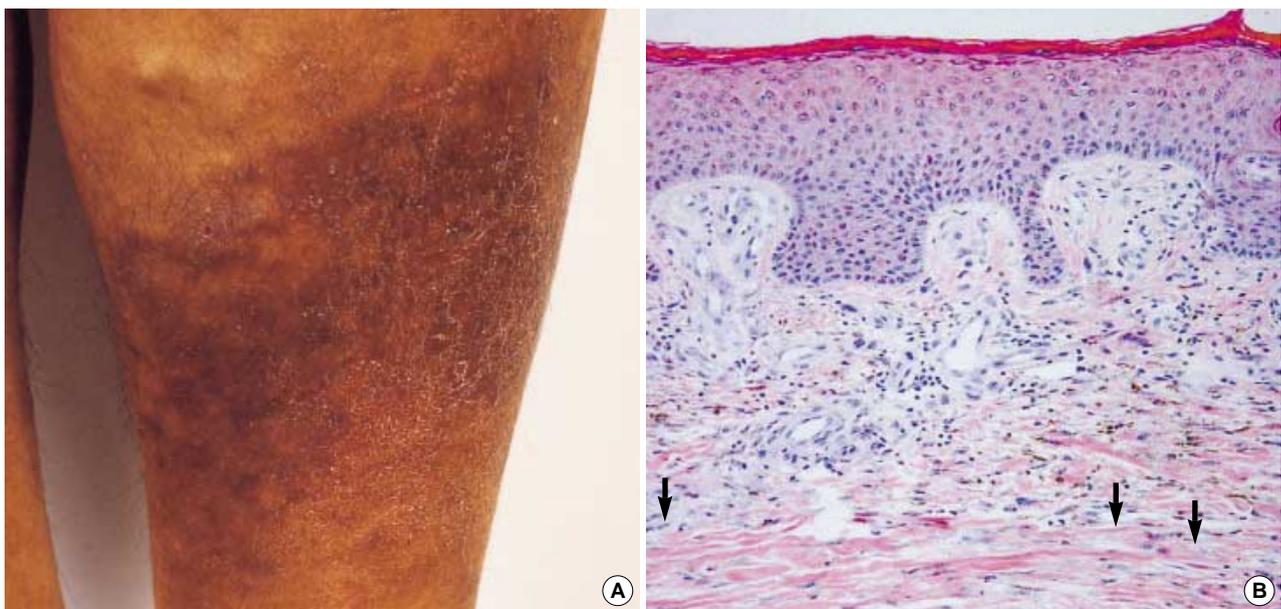


Fig. 1. Patient #9. (A) Scaly, dark brown colored skin eruptions are observed on the left lower leg with varicose vein. (B) Fibrosis and new blood vessels are seen within upper dermis. Abundant hemosiderin deposits (arrows) showing dark yellow granules are also visible (H&E, $\times 100$).

Histopathologic findings

All biopsy specimens showed a combination of the following histopathological characteristics (1, 3) of stasis dermatitis (Fig. 1B): 1) epidermal changes (hyperkeratosis, parakeratosis, and acanthosis), 2) proliferation of superficial dermal vessels, 3) fibrosis of the reticular dermis, and 4) hemosiderin depositions in dermis. Five out of 20 patients (#3, #4, #10, #13, #18) showed incontinence of melanin pigments of a varying degree (depth=0.12-0.47 mm) in the papillary der-

mis (Fig. 2).

Detection of dermal melanocytes containing melanin pigments

Two cases out of 20 patients (3 skin biopsy specimens from 25 samples) revealed dermal melanocytes containing melanin pigments (Fig. 3-5). Skin biopsy samples from patient #1 (M, 25) and #9 (M, 33) showed melanin pigments in Fontana-Masson stain (Fig 3A, 4A), melanocytes in immunostaining

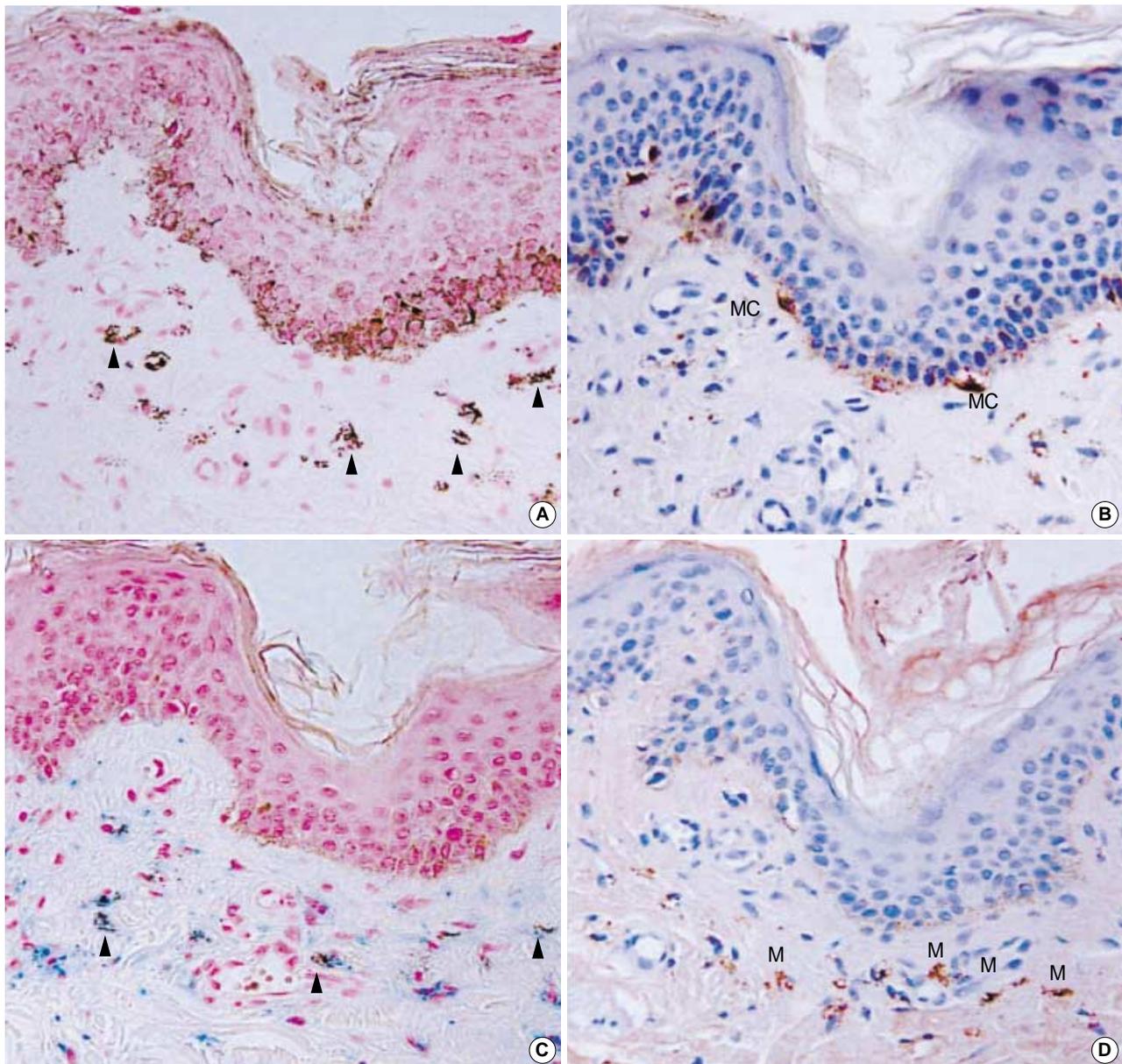


Fig. 2. Patient #3. Incontinence of melanin pigments is observed (depth=0.28 mm) ($\times 200$). (A) Melanin pigments (arrowheads) in upper dermis are stained black by Fontana-Masson stain. (B) The melanocytes (MC) in epidermis are stained red by immunohistochemical staining to GP 100, but melanin is not stained (brown). (C) Iron (Fe^{3+}) is stained blue in upper dermis, but melanin (arrowheads) is not stained. (D) The cytoplasm of macrophages (M) is stained red by immunohistochemical staining to CD68 (lysosomal marker), suggesting the phagocytosis of hemosiderin and melanin.

to GP 100 (Fig. 3B, 4B), hemosiderin pigments in Prussian blue stain (Fig. 3C, 4C) and siderophages in immunostaining to CD68 (Fig. 3D, 4D) in dermis. However, there was no clinical differences such as skin color, and location of the lesion between 2 cases showing dermal melanocytes and others. All patients showed hemosiderin and hemosiderin-laden macrophages known as siderophages in dermis. Hemosiderin pigments were not stained in Turnbull blue reaction for Fe^{2+} but were stained blue in Prussian blue reaction for

Fe^{3+} . In addition, triple stain (immunohistochemical staining to CD68 combined with Fontana-Masson stain, and Perls' stain) demonstrated the coexistence of melanin, melanocytes, hemosiderin, and siderophages in one section (Fig. 5).

DISCUSSION

Positive reaction by immunohistochemical staining using

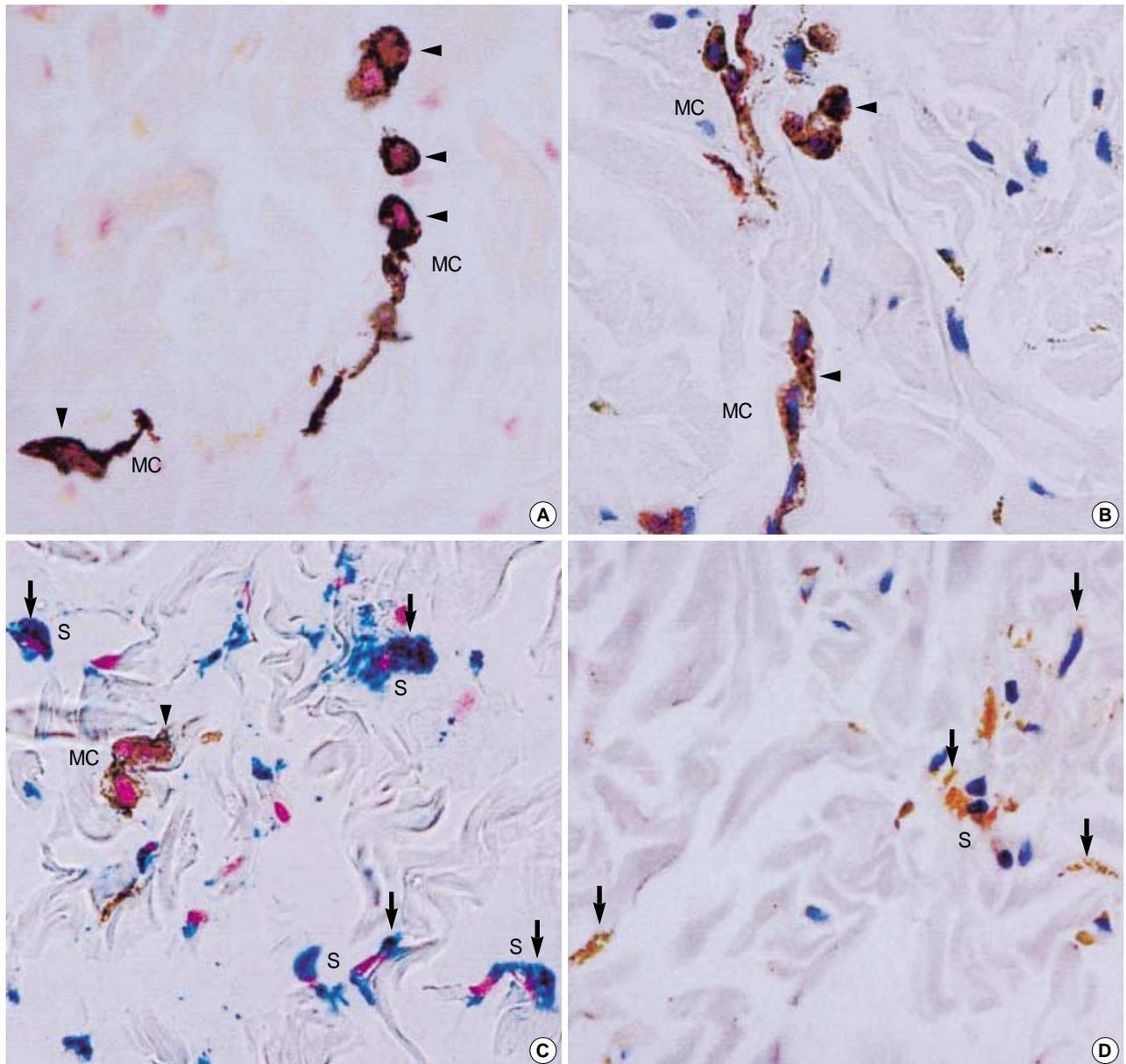


Fig. 3. Patient #1. Melanocytes are clearly seen in the deep dermis (depth=1.1 mm) in stasis dermatitis ($\times 400$). (A) Melanin (arrowheads) produced by melanocytes (MC) is stained black by Fontana-Masson stain. (B) The cytoplasm of the melanocyte (MC) is stained red but partially stained dark by melanin (arrowheads) within the melanocytes by immunohistochemical staining to GP 100. (C) Hemosiderin (arrows) within siderophages (S) is stained blue by Perls' stain for Fe^{3+} (Prussian blue reaction). Note that brown melanins (arrowheads) within the melanocytes (MC) are not stained. (D) The cytoplasm of siderophages (S) is stained red but iron (arrows) is not stained by immunohistochemical staining to CD68.

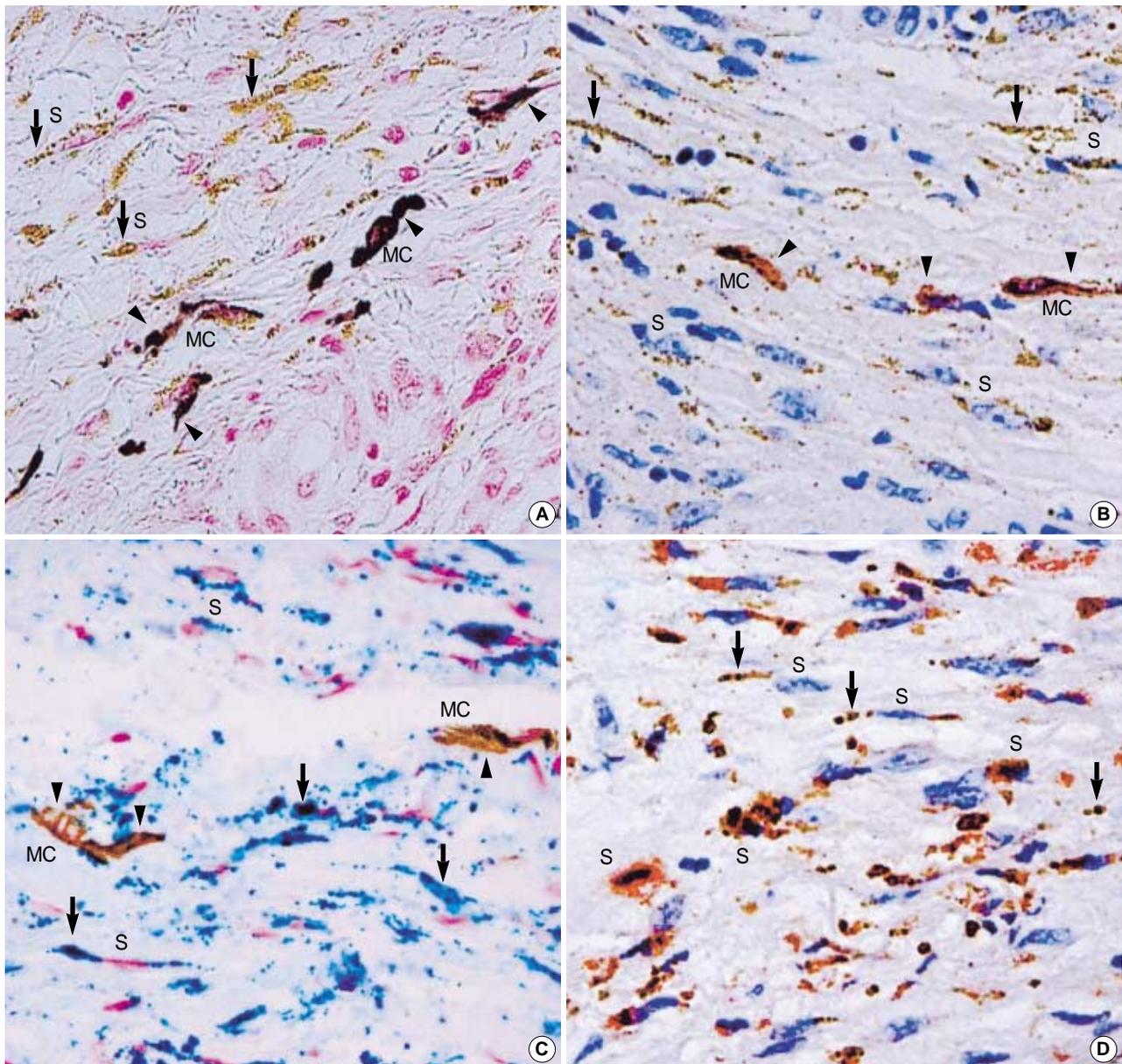


Fig. 4. Patient #9. Melanocytes as well as siderophages are clearly seen in the deep dermis (depth=0.7 mm) of stasis dermatitis ($\times 400$). (A) Melanin (arrowheads) produced by melanocytes (MC) is stained black in Fontana-Masson stain. Note that the golden-yellow colored iron (arrows) is visible within the siderophages (S), but is not stained. (B) The melanocytes (MC) containing melanin (arrowheads) are stained red by immunohistochemical staining to GP 100. The dark yellow colored iron (arrows) is not stained. (C) Hemosiderin (arrows) in free form or within siderophages (S) is stained blue by Perls' stain. Note that wavy melanocytes (MC) containing brown melanin (arrowheads) are not stained. (D) The cytoplasm of siderophages (S) is stained red by immunohistochemical staining to CD68. Some of the siderophages contain brown colored iron (arrows, not stained) in their cytoplasm.

NKI-beteb antibodies to GP 100 indicates the presence of (pre) melanosome in the cytoplasm. The NKI-beteb antibody is known to be specific to melanoma cells, nevocellular nevi, and normal melanocytes (9, 10). We employed this immunostaining method in pigmented diseases including melasma (12), nevus of Ota (13), and in image analysis of pigmented disorder (14) by using formalin-fixed paraffin embedded tissue sections. The present study demonstrated the presence

of dermal melanocytes actively synthesizing melanin in two cases; patients #1 (Fig. 3) and #9 (Fig. 4, 5). These findings were different from the incontinence of melanin pigment appeared in 5 cases of this study (patients #3, #4, #10, #13, #18), where melanin pigments were scattered in the upper dermis mainly within the melanophages instead of melanocytes (Fig. 2). There have been no reports demonstrating the dermal melanocytes in stasis dermatitis. According to the

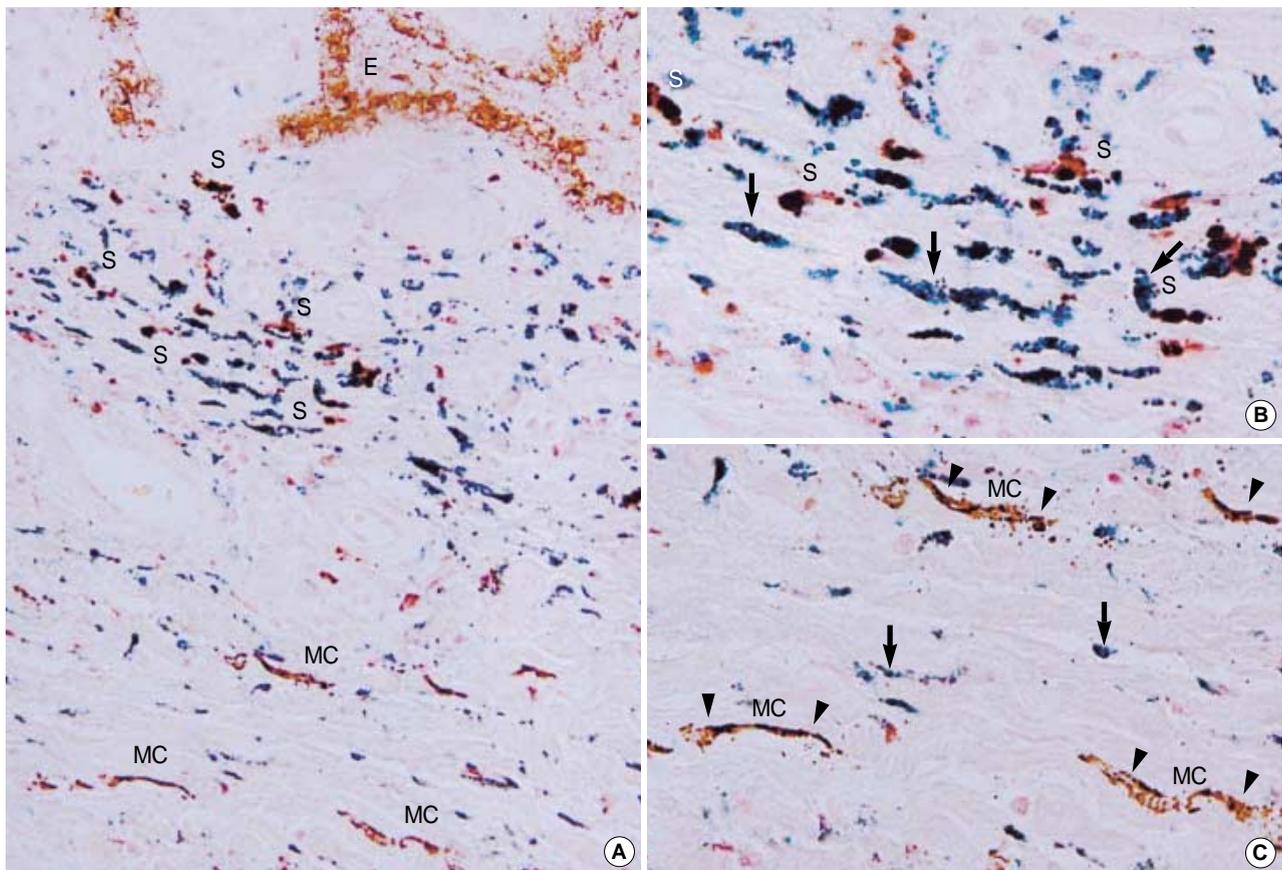


Fig. 5. Patient #9. (A) The coexistence of melanin (within melanocytes), iron, and siderophages in the upper and mid dermis is shown on a single section by triple stain (immunohistochemical staining to CD68 combined with Fontana-Masson stain, and Perls' stain). ($\times 200$). (B) Iron (arrows) in free form or within siderophages (S) is stained blue. The cytoplasm of siderophages is stained red ($\times 400$). (C) Melanin pigments (arrowheads) within the melanocytes (MC) are stained brown similar with those of the basal layer of the epidermis (E) ($\times 400$).

reports about postsclerotherapy hyperpigmentation (15, 16), the skin pigmentation of chronic venous insufficiency is known to be associated with hemosiderin deposits and/or increased melanin production. However, none of the patients ($n=20$) in this study received previous sclerotherapy before skin biopsy at our dermatologic clinic.

Iron and other metal ions including copper and zinc are known to be the non-melanosome regulatory factors for melanogenesis (17). Hereditary hemochromatosis, a disease with a characteristic increase in parenchymal iron, provides some clues for a certain role of iron in melanogenesis, which is unclear yet. Milman (18) reported, in a study of 179 patients with hereditary hemochromatosis, that the skin biopsy samples were positive for hemosiderin in 35 (63%) out of 56 patients with skin pigmentation, and positive for melanin in 26 (58%) out of 45 assessable patients. But there was no mention about the origin and localization of melanin. There was no mention about dermal melanocytes, either. Tsuji (19) injected iron (totaling 7.5 mg per mouse in three doses) into 20 hairless mice intraperitoneally to find out the histological factor(s) determining clinical pigmentation. He assumed

that the indeterminate dendritic cells in the epidermis would be activated and would produce melanin, but failed to prove the production of melanin from epidermis (by the epidermal dendritic cells) or dermis. As expected, hemosiderin granules were scattered extracellularly between collagen bundles as well as within dermal macrophages, Langerhans cells, or indeterminate dendritic cells of epidermis. The present study showed incontinence of melanin pigment in 5 cases, which indicates that melanin pigments from epidermis could contribute to the cutaneous pigmentation in stasis dermatitis and that iron might have a role in production of melanin.

Melanocytes are normally present in epidermis, not in dermis. However, in this study, dermal melanocytes were observed in two patients. In order to understand the pathogenesis of "unusual dermal melanocytes" in stasis dermatitis, the mechanism of other dermatologic diseases such as nevus of Ota, acquired bilateral nevus of Ota-like macule (ABNOM), nevus of Ito, Mongolian spots, and dermal melanocytic hamartoma (13, 20, 21) should be reviewed. Hori et al. (21) proposed the following possibilities for the pathogenesis of ABNOM; 1) the dropping off of epidermal melanocytes, 2)

the migration of hair bulb melanocytes, 3) the reactivation of preexisting dermal melanocytes, or 4) triggering of latent dermal melanocytosis by dermal inflammation. A similar mechanism is suspected for the development of dermal melanocytes in stasis dermatitis.

Stasis dermatitis develops as a complication of impaired venous return, and shows the vascular proliferation, red blood cells extravasation, and hemosiderin deposition histologically. Inflammatory cytokines or growth factors have been considered responsible for the pathogenesis of venous leg ulceration (22, 23). In addition to the mechanism suggested by Hori et al. (21), we also suggest that iron (hemosiderin), various cytokines or growth factors derived from vascular inflammation might have an unproven effect on possible pluripotent stem cells which are involved in the development of dermal melanocytes.

In conclusion, the results of this study propose the possible role of iron, inflammatory cytokines or growth factors in the development of dermal melanocytes in stasis dermatitis. Further studies should be conducted to determine the exact role of iron and various inflammatory cytokines or growth factors and to identify the role of pluripotent stem cells in the pathogenesis of hyperpigmentation in stasis dermatitis.

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